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Attorney Docket No. 22596-536

09/17/02 JC970 U.S. PTO

JC997 U.S. PTO 60/411478

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c)

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TITLE OF THE INVENTION

Novel Lapacho Compounds and Methods of Use Thereof

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ENCLOSED APPLICATION PARTS

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The invention was made by an agency of the United States Government or under a contract with an agency o. the United States Government:

☐ No.

☐ Yes

METHOD OF PAYMENT

☒ A check in the amount of **$160.00** is enclosed to cover the filing fees of the Provisional application.

☐ The Commissioner is hereby authorized to charge **$160.00** to cover the filing fees of the Provisional application to Deposit Account No. 50-0311, Reference No. 22596-536

☒ The Commissioner is hereby authorized to charge additional fees or credit any overpayment to Deposit Account No 50-0311, Reference No. 22596-536.

Respectfully submitted,

Dated: September 17, 2002

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PATENT TRADEMARK OFFICE

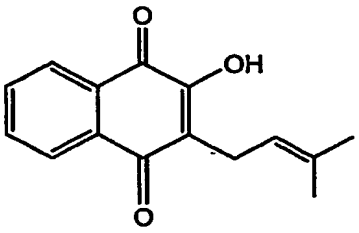
Express Mail Label No.: EL947114036US
Date of Deposit: September 17, 2002
Attorney Docket No. 22596-536

NOVEL LAPACHO COMPOUNDS AND METHODS OF USE THEREOF

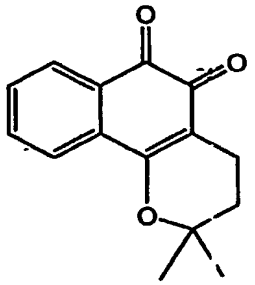
BACKGROUND OF THE INVENTION

Lapacho ("pau d'arco", "ipê-roxo", "taheebo") is a commercial natural product obtained from the bark of *Tabebuia* trees, and in particular from *T. impetiginosa* (Martius ex DC.) Standley (Binoniaceae), which are found in the rainforests throughout Central and South America. Lapacho has been used as a folk medicine for many years, in particular for the treatment of cancer (Hartwell, J. L., Lloydia, 31, 71-170, 1968) and disorders of the immune system, including psoriasis (Jones, K., Pau D'arco: Immune Power from the Rain Forest; Healing Arts Press; Rochester, Vermont, 1995).

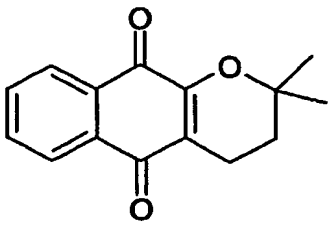
The occurrence of naphthoquinones in various members of the genus *Tabebuia* has been widely reported (Burnett, A.R., et. al., J. Chem. Soc., C, 2100-2104, 1967; Rao, M.M, et. al, J. Nat. Prod., 45, 600-604, 1982; Girard, M., et. al., J. Nat. Prod., 51, 1023-1024, 1988; and Diaz, F., et. al., J. Nat. Prod., 59, 423-424, 1996). The best known of these compounds are lapachol, alpha-lapachone (α-lapachone) and beta-lapachone (β-lapachone), which have the following chemical structures:



Lapachol



Beta-Lapachone



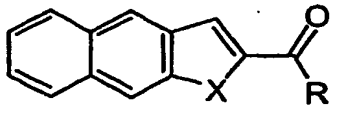
Alpha-Lapachone

Although all three of these compounds have been reported to have antiproliferative activity, β-lapachone, in particular, has demonstrated significant antineoplastic activity against a wide spectrum of human cancer cell lines at concentrations typically in the range of 1-10 μM (IC_{50}). For example, the cytotoxicity of β-lapachone has been demonstrated in transformed cell lines derived from patients with promyelocytic leukemia (Planchon *et al.*, *Cancer Res.*, 55 (1996) 3706), prostate (Li, C.J., *et al.*, *Cancer Res.*, 55 (1995) 3712), malignant glioma (Weller, M. *et al.*, *Int. J. Cancer*, 73 (1997) 707), hepatoma (Lai, C.C., *et al.*, *Histol Histopathol*, 13 (1998) 8), colon (Huang, L., *et al.*, *Mol Med*, 5, (1999) 711), breast (Wuertzberger, S.M., *et al.*, *Cancer Res.*, 58 (1998) 1876), ovarian (Li, C.J. *et al.*, *Proc. Natl. Acad. Sci. USA*, 96(23) (1999) 13369-74), pancreatic (Li, Y., *et al.*, *Mol Med*, 6 (2000) 1008; Li, Y.Z., *Mol Med*, 5 (1999) 232), and multiple myeloma cell lines, including drug-resistant lines (Li, Y., *Mol Med*, 6 (2000) 1008). No cytotoxic effects were observed on normal fresh or proliferating human PBMC (Li, Y., *Mol Med*, 6 (2000) 1008).

Other lapacho-derived compounds have been shown to have antiproliferative activity. Eight compounds, representing the most common constituents of the inner bark of *T. impetiginosa* and including lapachol, α-lapachone and β-lapachone, were evaluated for antiproliferative and cytotoxic activity in the nontransformed human keratinocyte cell line HaCaT, a model for the highly proliferative epidermis characteristic of psoriasis (Müller, K., *et al.*, *J. Nat. Prod.* 62 (1999) 1134-1136). While lapachol and α-lapachone were relatively inactive in this model, β-lapachone and several naphtho[2,3-b]furan diones displayed inhibition of keratinocyte growth comparable to the antipsoriatic drug anthralin. These findings encourage the design and synthesis of new lapacho compounds and their evaluation for antiproliferative activity in a variety of biological systems.

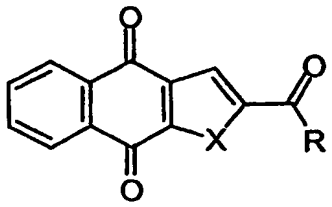
DESCRIPTION OF THE INVENTION

The present invention concerns new synthetic lapacho derivatives of Formula I:



I

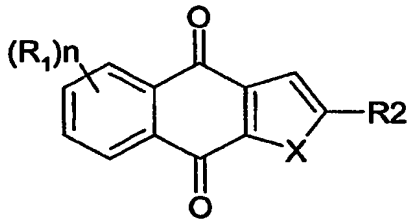
or Formula II:



II

or a pharmaceutically acceptable salt thereof, wherein X is O or S; and R is straight-chained or branched alkyl containing 1-6 carbons, aryl, substituted aryl (substituted, for example, with: hydroxyl, alkoxy, alkyl, nitro, halogen carboxyl, carboxyalkyl), or straight-chained or branched alkylaryl.

The present invention also concerns new synthetic lapacho analogs of Formula III:



III

or a pharmaceutically acceptable salt thereof, wherein X is O or S; R_1 is independently at each incidence hydrogen, hydroxyl, alkoxyl, alkyl of 1-6 carbons, nitro, halogen, carboxyl or carboxyalkyl; R_2 is hydrogen, acyl, straight-chained or branched alkyl containing 1-6 carbons or carboxyalkyl; and n is 0, 1 or 2.

Preferred compounds of Formula I are those in which X is S and R is aryl or substituted aryl.

Preferred compounds of Formula II are those in which X is O or S and R is alkyl, aryl or mono- or di-substituted aryl.

Preferred compounds of Formula III are those in which X is S, R_1 is hydroxyl or alkylcarbonyl, R_2 is hydrogen, and n is 1 or 2.

The most preferred compounds of the invention are shown in Figures 1, 2 and 3.

The present invention also provides pharmaceutical formulations comprising a compound of Formula I, II or III in combination with at least one pharmaceutically acceptable excipient or carrier.

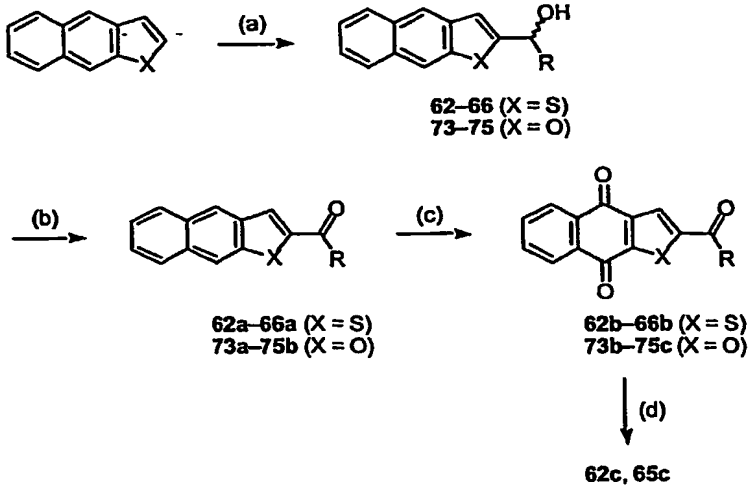
The present invention also provides a method for the treatment of cell proliferative disorders in mammals comprising administering to a mammal in need of such treatment an effective amount of a compound of Formula I, II or III. The invention further provides the use of a compound of Formula I, II or III for the preparation of a medicament useful for the treatment of a cell proliferative disorder.

As used herein, the term "cell proliferative disorder" refers to conditions in which the unregulated and/or abnormal growth of cells can lead to the development of an unwanted condition or disease, which can be cancerous or non-cancerous, for example a psoriatic condition. As used herein, the term "psoriatic condition" refers to disorders involving keratinocyte hyperproliferation, inflammatory cell infiltration, and cytokine alteration.

In addition to psoriatic conditions, the types of proliferative diseases which may be treated using the compositions of the present invention are epidermic and dermoid cysts, lipomas, adenomas, capillary and cutaneous hemangiomas, lymphangiomas, nevi lesions, teratomas, nephromas, myofibromatosis, osteoplastic tumors, and other dysplastic masses and the like.

The process used for the preparation of most preferred compounds of Formula I and II is shown in Scheme 1.

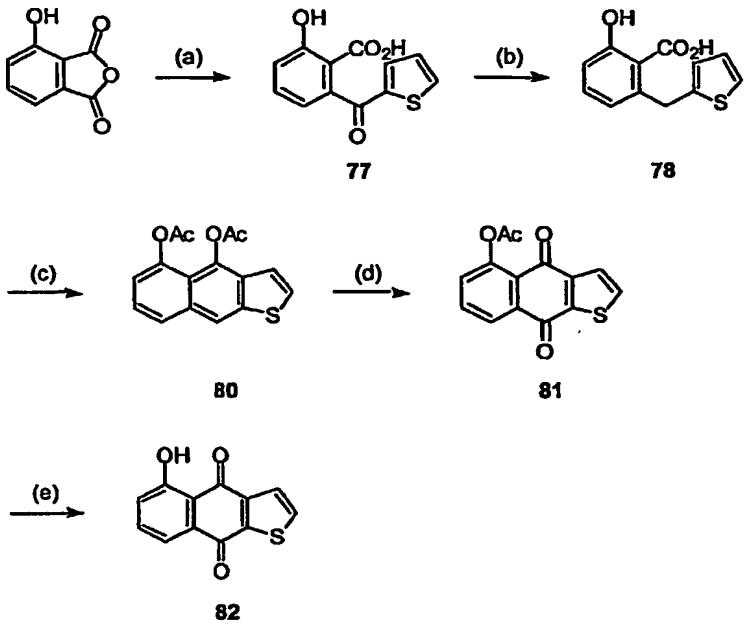
Scheme 1



Reagents: (a) *sec*-BuLi, tetramethylethylenediamine, ether, - 78 °; (b) MnO_2, CH_2Cl_2; (c) CrO_3, HOAc; (d) BBr_3, CH_2Cl_2. R and X are defined in Figures 1 and 2.

The process used for the preparation of most preferred compounds of Formula III is shown in Scheme 2.

Scheme 2



Reagents: (a) thiophene, $AlCl_3$, CH_2Cl_2; (c) Zn, NH_3, Δ; (c) Ac_2O, HOAc, $ZnCl_2$, Δ; (d) CrO_3, HOAc; (e) 6 N-NaOH, Δ.

Compounds of the present invention have demonstrated potent antiproliferative activity against the nontransformed human keratinocyte line HaCaT, as demonstrated by reduction in cell number over time as compared to control plates. Anthralin, an antipsoriatic drug, was used as a positive control. Antiproliferative activity was measured directly by counting the dispersed cells under a phase-contrast microscope. Table 1 shows the concentrations of the compounds required to inhibit 50% of cell growth (IC_{50}). The cytotoxicity of naphthoquinones has been thought to result, at least in part, from reactive oxygen species, generated during redox cycling between the quinine and reduction products (Munday, R., Free Radic. Biol. Med., 22, 689-695, 1997), which cause peroxidative damage to membrane lipids. To assess the correlation of keratinocyte growth

inhibition with membrane damage, the release of lactate dehydrogenase from the treated cells was also quantitated.

Table 1. Antiproliferative activity and cytotoxicity against HaCaT cells by synthetic lapacho analogs

Compound	X	R	R1	R2	AA[a] IC_{50} (μM)	LDH[b] (mU)
Compounds of Formula I						
73a	O	Me	NA	NA	> 5	ND
74a	O	Ph	NA	NA	1.9	142
75a	O	3,4-$(OMe)_2$-Ph	NA	NA	> 5	ND
63a	S	Me	NA	NA	5.0	ND
64a	S	Ph	NA	NA	0.3	122
62a	S	4-OMe-Ph	NA	NA	> 5	ND
65a	S	3,4-$(OMe)_2$-Ph	NA	NA	> 5	ND
66a	S	4-NO_2-Ph	NA	NA	> 5	ND
Compounds of Formula II						
6	O	Me	NA	NA	0.5	331
74b	O	Ph	NA	NA	0.7	222
75b	O	3,4-$(OMe)_2$-Ph	NA	NA	2.5	250
63b	S	Me	NA	NA	0.3	134
64b	S	Ph	NA	NA	1.7	ND
62b	S	4-OMe-Ph	NA	NA	> 5	ND
65b	S	3,4-$(OMe)_2$-Ph	NA	NA	0.8	137
62c	S	4-OH-Ph	NA	NA	2.7	123
65c	S	3,4-$(OH)_2$-Ph	NA	NA	1.5	118
66a	S	4-NO_2-Ph	NA	NA	4.0	ND
Compounds of Formula III						
7	O	NA	8-OH	COMe	0.3	346
45	S	NA	H	H	> 5	222
81	S	NA	5-OCOMe	H	1.4	160
82	S	NA	5-OH	H	1.0	117
Controls						
α-lapachone	NA	NA	NA	NA	10	ND
β-lapachone	NA	NA	NA	NA	0.7	329
anthralin	NA	NA	NA	NA	0.7	294
vehicle	NA	NA	NA	NA	NA	135

[a]Antiproliferative activity against HaCaT cells. Inhibition of cell growth was significantly different with respect to that of the control, $N = 3$, $p < 0.05$. [b]Activity of LDH (mU) release in HaCaT cells after treatment with 2 μM test compound, $N = 3$, SD < 10%, $p < 0.05$. NA = not applicable. ND = not determined.

As shown in Table 1, treatment of HaCaT cells with anthralin was effective at inhibition of proliferation (IC_{50} = 0.7 μM) but caused substantial cellular damage, with LDH release

significantly higher than vehicle controls. Similarly, β-lapachone and the 2-acetylated naphtho[2,3-b]furan-4,9-diones (compounds 6 and 7) inhibited cell proliferation but caused significant LDH release as compared to vehicle. However, several of the thiophene analogs (compounds **63a, 64b, 65b, 65c, 81** and **82**) inhibited cell proliferation at concentrations comparable to β-lapachone and the furan analogs but without significant elevation of LDH release over the vehicle control.

The similarity of their antiproliferative response to that of β-lapachone indicates that the present synthetic lapacho derivative compounds may be expected to show wide anticancer activity. β-lapachone has been shown to be active against breast cancer, leukemia, lung cancer, ovarian cancer, brain cancer, liver cancer, prostate cancer, and colorectal cancer. The lapacho derivatives of the present invention would also be effective in treating these disorders. These treatments may be accomplished utilizing the present lapacho derivative compounds (Formula I, II or III) alone or in combination with prior art chemotherapy agents or with radiation therapy. In a preferred embodiment the present lapacho derivative compounds are used for the treatment of cancer as a preventative drug by preventing cancer cell formation.

A variety of cancer cell lines are contemplated to determine the effectiveness of the novel lapacho derivatives of the present invention, including SK-OV-3 and OVCAR-3 human ovarian carcinoma cells; SW-480, HT-29 and HCT-116 human colon carcinoma cells; MCF-7 and MDA-MB-231 human breast carcinoma cells; MIA PACA-2 and BXPC-3 human pancreatic carcinoma cells; NCI-H226 and A549 human lung carcinoma cells; and DU-145 and PC-3 human prostate cancer cells. Since β-lapachone induces apoptosis only in cancer cell lines and not in normal cells (Li.,Y, et. al., PNAS, in press) the present compounds will also be tested in a panel of normal cell lines including NCM 460 normal colonic epithelial cells and MCF 10A normal breast epithelial cells.

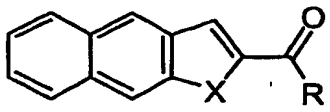
One potential effect of the present lapacho derivatives is induction of E2F1. Experiments have shown that β-lapachone induces sustained E2F1 activity in nuclei of cancer cells but not in normal cells, resulting in the arrest of cancer cells in G1 and/or S phase. The present lapacho derivatives would also be effective in sustaining E2F1 activity, thus causing G1 and/or S phase arrest. Furthermore, the present lapacho derivatives would have no significant toxic effects on normal cells.

The results of experiments with β-lapachone and similar chemical compounds have shown that the present lapacho derivatives would have a strong apoptotic effect on a variety of human cancer cells and that they can inhibit growth of other human cancer cells. The lack of toxic effects on normal cells at the concentrations needed for effectiveness against the cancer cells indicates that the present lapacho derivatives are very valuable chemotherapeutic reagents. It could be applied in many of the well-known methods currently used for chemotherapeutic treatment. For example, it may be injected directly into tumors, injected into the blood stream or body cavities or taken orally or applied through the skin with patches. The dose chosen should be sufficient to constitute effective treatment but not so high as to cause unacceptable side effects. The state of the cancer and the health of the patient should preferably be closely monitored during and for a reasonable period after treatment.

CLAIMS

We claim:

1. A compound of formula I:



I

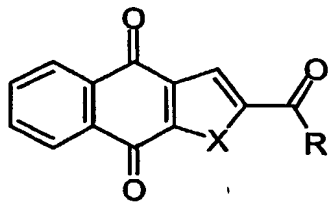
or a pharmaceutically acceptable salt thereof or a regioisomeric mixture thereof, wherein X is O or S; and R is straight-chained or branched alkyl having 1-6 carbons, aryl, substituted aryl, or straight-chained or branched alkylaryl.

2. A compound according to claim 1, wherein X is S, and R is aryl or substituted aryl.

3. A compound according to claim 1 or 2, wherein said substituted aryl may be substituted with hydroxyl, alkoxy, alkyl, nitro, halogen carboxyl or carboxyalkyl.

4. A compound according to claim 1, wherein X is S, and R is phenyl.

5. A compound of formula II:



II

or a pharmaceutically acceptable salt thereof or a regioisomeric mixture thereof wherein X is O or S; and R is straight-chained or branched alkyl having 1-6 carbons, aryl, substituted aryl, or straight-chained or branched alkylaryl.

6. A compound according to claim 5, wherein X is O or S, and R is alkyl, aryl or mono- or di-substituted aryl.

7. A compound according to claim 5 or 6, wherein said substituted aryl may be substituted with hydroxyl, alkoxy, alkyl, nitro, halogen carboxyl or carboxyalkyl.

8. A compound according to claim 5, wherein X is O, and R is phenyl.

9. A compound according to claim 5, wherein X is O, and R is 3,4-dimethoxyphenyl.

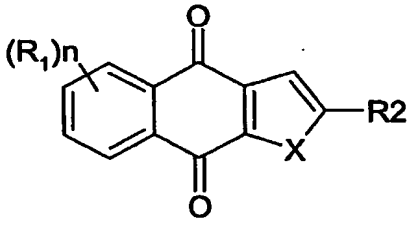
10. A compound according to claim 5, wherein X is S, and R is phenyl.

11. A compound according to claim 5, wherein X is S, and R is 3,4-dimethoxyphenyl.

12. A compound according to claim 5, wherein X is S, and R is 4-hydroxyphenyl.

13. A compound according to claim 5, wherein X is S, and R is 3,4-dihydroxyphenyl.

14. A compound of formula III:



III

or a pharmaceutically acceptable salt thereof or a regioisomeric mixture thereof, wherein X is O or S; R_1 is independently at each incidence hydrogen, hydroxyl, alkoxyl, alkyl having 1-6 carbons, nitro, halogen, carboxyl or carboxyalkyl; R_2 is hydrogen, acyl, straight-chained or branched alkyl of 1-6 carbons or carboxyalkyl; and n is 0, 1 or 2.

15. A compound according to claim 14, wherein X is S, R_1 is hydroxyl or alkylcarbonyl, R_2 is hydrogen, and n is 1 or 2.

16. A compound according to claim 14, wherein X is S, R_1 is 5-carboxymethyl, R_2 is hydrogen, and n is 1.

17. A compound according to claim 14, wherein X is S, R_1 is 5-hydroxyl, R_2 is hydrogen, and n is 1.

18. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 1 in combination with a pharmaceutically acceptable carrier.

19. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 4 in combination with a pharmaceutically acceptable carrier.

20. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 5 in combination with a pharmaceutically acceptable carrier.

21. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to any one of claims 8-13 in combination with a pharmaceutically acceptable carrier.

22. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 14 in combination with a pharmaceutically acceptable carrier.

23. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claims 16 or 17 in combination with a pharmaceutically acceptable carrier.

24. A method of treating or preventing cell proliferative disorders comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition that induces sustained elevation of E2F activation in tumor cells without affecting E2F levels in normal cells.

25. The method according to claim 24, comprising the pharmaceutical composition of claim 18.

26. The method according to claim 24, comprising the pharmaceutical composition of claim 19.

27. The method according to claim 24, comprising the pharmaceutical composition of claim 20.

28. The method according to claim 24, comprising the pharmaceutical composition of claim 21.

29. The method according to claim 24, comprising the pharmaceutical composition of claim 22.

30. The method according to claim 24, comprising the pharmaceutical composition of claim 23

31. A method of treating cancer comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 18.

32. A method of treating cancer comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 19.

33. A method of treating cancer comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 20.

34. A method of treating cancer comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 21.

35. A method of treating cancer comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 22.

36. A method of treating cancer comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 23.

37. A method of treating psoriasis comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 18.

38. A method of treating psoriasis comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 19.

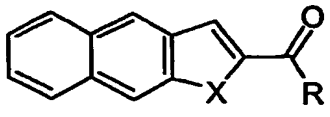
39. A method of treating psoriasis comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 20.

40. A method of treating psoriasis comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 21.

41. A method of treating psoriasis comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 22.

42. A method of treating psoriasis comprising administering to a mammal in need thereof a therapeutically effective amount of a pharmaceutical composition according to claim 23.

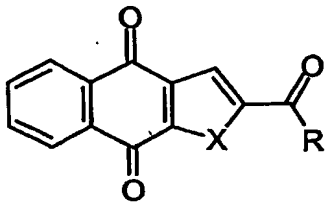
Figure 1



I

Compound	X	R
73a	O	Me
74a	O	Ph
75a	O	3;4-$(OMe)_2$-Ph
63a	S	Me
64a	S	Ph
62a	S	4-OMe-Ph
65a	S	3,4-$(OMe)_2$-Ph
66a	S	4-NO_2-Ph

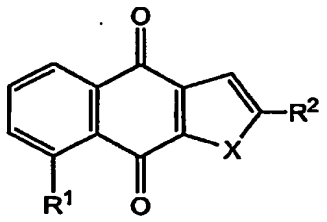
Figure 2



II

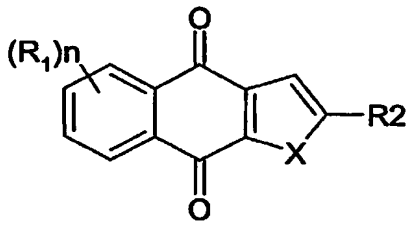
Compound	X	R
6	O	Me
74b	O	Ph
75b	O	3,4-$(OMe)_2$-Ph
63b	S	Me
64b	S	Ph
62b	S	4-OMe-Ph
65b	S	3,4-$(OMe)_2$-Ph
62c	S	4-OH-Ph
65c	S	3,4-$(OH)_2$-Ph
66a	S	4-NO_2-Ph

Figure 3A



Compound	X	R^1	R^2
7	O	OH	COMe

Figure 3B



III

Compound	X	R^1	R^2
45	S	H	H
81	S	5-OCOMe	H
82	S	5-OH	H